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(54) Title: MASS SPECTROMETRIC DETECTION OF POLYPEPTIDES

(57) Abstract

A process for determining the identity of a target polypeptide using mass spectroscopy is provided. Depending on the target polypeptide to be identified, a process as disclosed can be used, for example, to diagnose a genetic disease or chromosomal abnormality, a predisposition to a disease or condition, or infection by a pathogenic organism; or for determining identity or heredity. Kits for performing the disclosed processes also are provided.

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WHAT IS CLAIMED IS:

1. A process for determining the identity of a target polypeptide, comprising the steps of:

a) obtaining the target polypeptide by *in vitro* translation, or by *in vitro* transcription followed by translation, of a nucleic acid encoding the target polypeptide;

b) determining the molecular mass of the target polypeptide by mass spectrometry; and

c) comparing the molecular mass of the target polypeptide with the molecular mass of a corresponding known polypeptide, thereby determining the identity of the target polypeptide.

2. A process for determining the identity of a target polypeptide, comprising the steps of:

a) determining the molecular mass of the target polypeptide by mass spectrometry; and

b) comparing the molecular mass of the target polypeptide with the molecular mass of a corresponding known polypeptide, thereby determining the identity of the target polypeptide.

3. The process of claim 1, wherein the nucleic acid encoding the target polypeptide is RNA, and wherein the target polypeptide is obtained by *in vitro* translation.

4. The process of claim 1, wherein an RNA encoding the target polypeptide is prepared by *in vitro* transcription of the nucleic acid encoding the target polypeptide, and wherein the target polypeptide is obtained by *in vitro* translation of the RNA.

5. The process of claim 1, further comprising amplifying the nucleic acid encoding the target polypeptide.

6. The process of claim 5, wherein the amplifying is performed using a forward primer and a reverse primer.

7. The process of claim 5, wherein the amplifying is performed using a primer comprising a nucleotide sequence encoding a regulatory element selected from the group consisting of a ribosome binding site, a START codon and a transcription start signal, wherein, following amplification, the regulatory element is operably linked to the nucleic acid encoding the target polypeptide.

8. The process of claim 5, wherein the amplifying is performed using a primer comprising a nucleotide sequence encoding an RNA polymerase promoter, wherein, following amplification, the promoter is operably linked to the nucleic acid encoding the target polypeptide.

9. The process of claim 8, wherein the RNA polymerase promoter is selected from the group consisting of SP6 promoter, T3 promoter, and T7 promoter.

10. The process of claim 1, wherein the nucleic acid further comprises an operably linked exogenous nucleotide sequence encoding a regulatory element selected from the group consisting of an RNA polymerase promoter, a ribosome binding site, a START codon, and a transcription start signal.

11. The process of claim 1, wherein the nucleic acid comprises a nucleotide sequence, or complement thereof, encoding a second polypeptide.

12. The process of claim 11, wherein the second polypeptide is a tag peptide.

13. The process of claim 12, wherein the tag peptide is selected from the group consisting of a myc epitope, a *Haemophilus influenza* hemagglutinin peptide, a polyhistidine sequence, a polylysine sequence, a polyarginine sequence, and glutathione-S-transferase.

14. The process of claim 1 or claim 2, wherein the target polypeptide comprises a tag.

15. The process of claim 14, wherein the tag is biotin or a derivative thereof.

16. The process of claim 14, wherein the tag is a tag peptide, which is conjugated to the target polypeptide.

17. The process of claim 3, wherein the *in vitro* translation is performed in a cell-free extract.

18. The process of claim 17, wherein the cell-free extract is a eukaryotic cell-free extract.

19. The process of claim 18, wherein the eukaryotic cell-free extract is selected from the group consisting of a reticulocyte lysate, a wheat germ extract, and a combination thereof.

20. The process of claim 4, wherein the *in vitro* transcription is performed in a cell-free extract, and wherein translation of the target polypeptide is performed in the same cell-free extract.

21. The process of claim 20, wherein the cell-free extract comprises a reticulocyte lysate.

22. The process of claim 20, wherein the cell-free extract is a prokaryotic cell-free extract.

23. The process of claim 22, wherein the prokaryotic cell-free extract is an *E. coli* cell-free extract.

24. The process of claim 23, wherein the cell-free extract is *E. coli* S30 cell-free extract.

25. The process of claim 1, wherein transcription or translation is performed *in vivo*.

26. The process of claim 25, which is performed in a host cell.

27. The process of claim 26, wherein the host cell is a bacterium.

28. The process of claim 1 or claim 2, wherein the target polypeptide is isolated prior to mass spectrometry.

29. The process of claim 28, wherein the target polypeptide is isolated be reaction with an antibody.

30. The process of claim 14, wherein the target polypeptide is isolated by reaction a reagent that interacts specifically with the tag.

31. The process of claim 30, wherein the tag is a tag peptide and the reagent is an antibody.

32. The process of claim 30, wherein the tag is a polyhistidine tag peptide and the reagent is a metal ion selected from the group consisting of nickel ions and cobalt ions, or wherein the tag is a polylysine or a polyarginine tag peptide and the reagent is selected from the group consisting of copper ions and zinc ions, wherein the reagent is chelated to a solid support.

33. The process of claim 30, wherein the tag is biotin or a derivative thereof and the reagent is selected from the group consisting of avidin and streptavidin.

34. The process of claim 1 or claim 2, wherein, prior to determining the molecular mass of the target polypeptide by mass spectrometry, the target polypeptide is immobilized on a solid support.

35. The process of claim 34, wherein the target polypeptide is immobilized to the solid support through a cleavable linker.

36. The process of claim 35, wherein the cleavable linker is selected from the group consisting of an acid cleavable linker and a photocleavable linker.

37. The process of claim 34, wherein the target polypeptide is immobilized by interacting specifically with a polypeptide of interest that is conjugated to the solid support.

38. The process of claim 34, wherein the solid support is selected from the group consisting of a support having a flat surface and a support having a surface with a structure.

39. The process of claim 1 or claim 2, wherein the mass spectrometry is selected from the group consisting of matrix assisted laser desorption ionization (MALDI), delayed extraction MALDI, continuous or pulsed electrospray, ionspray, thermospray, or massive cluster impact and a detection format selected from the group consisting of linear time-of-flight, reflectron time-of-flight, single quadrupole, multiple quadrupole, single magnetic sector, multiple magnetic sector, Fourier transform ion cyclotron resonance, ion trap, and combinations thereof.

40. The process of claim 1 or claim 2, wherein the mass spectrometry is matrix-assisted laser desorption/ionization time-of-flight spectrometry.

41. The process of claim 1 or claim 2, wherein the target polypeptide is encoded by an allelic variant of a polymorphic region of a chromosome in a subject.

42. The process of claim 41, wherein the polymorphic region is in a gene.

43. The process of claim 41, wherein the polymorphic region is not in a gene.

44. The process of claim 41, wherein the allelic variant is associated with a disease or condition, thereby indicating that the subject has or is at risk of developing the disease or condition.

45. The process of claim 44, wherein the disease or condition is associated with an abnormal number of nucleotide repeats in the allelic variant.

46. The process of claim 45, wherein the nucleotide repeats are trinucleotide repeats.

47. The process of claim 46, wherein the disease or condition is selected from the group consisting of Huntington's disease, prostate cancer, Fragile X syndrome type A, myotonic dystrophy type I, Kennedy disease, Machado-Joseph disease, dentatorubral and pallidolyusian atrophy, spino bulbar muscular atrophy and aging.

48. The process of claim 42, wherein the gene is selected from the group consisting of BRCA1, BRCA2, APC, dystrophin gene, β-globin, Factor IX, Factor VIIc, ornithine-d-amino-transferase, hypoxanthine guanine phosphoribosyl transferase, CFTR, p53, and a proto-oncogene.

49. The process of claim 41, wherein the allelic variant is due to a point mutation.

50. The process of claim 42, wherein the polymorphic region is associated with graft rejection and the process is for determining compatibility between a donor and a recipient of a graft.

51. The process of claim 50, wherein the polymorphic region is the major histocompatibility locus.

52. The process of claim 41, wherein the target polypeptide is encoded by a nucleic acid comprising nucleotide repeats and the process is for a use selected from the group consisting of genotyping the subject, forensic analysis, and paternity testing.

53. The process of claim 52, wherein genotyping is performed by quantifying the number of nucleotide repeats.

54. The process of claim 52, wherein the nucleotide repeats are dinucleotide, trinucleotide, tetranucleotide, or pentanucleotide repeats.

55. The process of claim 41, wherein the gene is a mitochondrial gene.

56. The process of claim 1 or claim 2, wherein the target polypeptide is obtained from an infectious organism.

57. The process of claim 56, wherein the infectious organism is selected from the group consisting of a virus, a bacterium, a fungus, and a protist.

58. A process for determining the identity of each target polypeptide in a plurality of target polypeptides, comprising the steps of:

a) obtaining a plurality of differentially mass modified target polypeptides;

b) determining the molecular mass of each differentially mass modified target polypeptide in the plurality by mass spectrometry; and

c) comparing the molecular mass of each differentially mass modified target polypeptide in the plurality with the molecular mass of a corresponding known polypeptide, thereby determining the identity of each target polypeptide in the plurality of target polypeptides.

59. The process of claim 58, wherein the target polypeptides are obtained by *in vitro* translation, or by *in vitro* transcription, followed by translation, of a nucleic acid encoding the target polypeptide.

60. The process of claim 58, wherein, prior to determining the molecular mass of each differentially mass modified target polypeptide by mass spectrometry, each target polypeptide is immobilized on a solid support.

61. The process of claim 60, wherein each target polypeptide is immobilized to the solid support through a cleavable linker.

62. The process of claim 61, wherein the cleavable linker is selected from the group consisting of an acid cleavable linker and a photocleavable linker.

63. The process of claim 60, wherein the solid support is selected from the group consisting of a support having a flat surface and a support having a surface with a structure.

64. The process of claim 60, wherein each target polypeptide is immobilized in an array to the solid support.

65. The process of claim 60, wherein each target polypeptide is immobilized due to its interacting specifically with a polypeptide of interest, wherein the polypeptide of interest is conjugated in an array to the solid support.

66. A kit for determining the identity of a target polypeptide by mass spectrometry, comprising:

a) reagents necessary for *in vitro* transcription or *in vitro* translation of the target polypeptide; and

b) instructions for determining the identity of the target polypeptide by mass spectrometry.

67. The kit of claim 66, further comprising a forward primer and a reverse primer, each capable of hybridizing to and amplifying a nucleic acid encoding the target polypeptide.

68. The kit of claim 67, wherein either the forward primer or the reverse primer comprises a nucleotide sequence, which, following amplification, encodes a regulatory element operably linked to the nucleic acid encoding the target polypeptide.

69. The kit of claim 68, wherein the regulatory element is selected from the group consisting of an RNA polymerase promoter, a ribosome binding site, a START codon, and a transcription start signal.

70. The kit of claim 66, further comprising a reagent for isolating the target polypeptide.

71. A method for screening for or identifying a subject having or predisposed to a disease or condition, comprising:

a) determining the molecular mass of a target polypeptide by mass spectrometry;

b) comparing the molecular mass of the target polypeptide with the molecular mass of a corresponding known polypeptide, thereby determining the identity of the target polypeptide, wherein:

the target polypeptide, or a nucleic acid encoding the target polypeptide, is obtained from a biological sample obtained from the subject; and

the target polypeptide is a marker for the disease or condition.

72. The method of claim 71, wherein the target polypeptide is obtained from the biological sample.

73. The method of claim 71, wherein the target peptide is obtained by *in vitro* translation of a nucleic acid obtained from the subject, or by *in vitro* transcription of a nucleic acid encoding the target polypeptide and translation of RNA produced by the *in vitro* transcription.

74. The method of claim 71, wherein the sample is selected from the group consisting of a tissue sample, a cell sample and a biological fluid.

75. The method of claim 71, wherein the disease or condition is selected from the group consisting of Huntington's disease, prostate cancer, Fragile X syndrome type A, myotonic dystrophy type I, Kennedy disease, Machado-Joseph disease, dentatorubral and pallidolyusian atrophy, spino bulbar muscular atrophy, and aging.

76. The method of claim 71, wherein the nucleic acid comprises at least a portion of a gene selected from the group consisting of BRCA1, BRCA2, APC, dystrophin gene, β-globin, Factor IX, Factor VIIc, ornithine-d-amino-transferase, hypoxanthine guanine phosphoribosyl transferase, CFTR, p53, and a proto-oncogene.

77. The method of claim 71, wherein the disease or condition is caused by an organism selected from the group consisting of a virus, a bacterium, a fungus and a protist.

78. A process for determining the amino acid sequence of a polypeptide of interest using mass spectrometry, comprising the steps of:

a) contacting the polypeptide of interest with an agent that cleaves an amino acid from a terminus of the polypeptide to produce a cleaved amino acid and a deletion fragment;

b) subjecting the cleaved amino acid or the deletion fragment to mass spectrometry; and

c) repeating step a) and step b), as necessary, thereby determining the amino acid sequence of the polypeptide.

79. The process of claim 78, wherein the polypeptide of interest is obtained by *in vitro* translation of an RNA encoding the polypeptide, or by *in*

vitro transcription of a nucleic acid encoding the target polypeptide and translation of RNA produced by the *in vitro* transcription.

80. The process of claim 78, further comprising conditioning the polypeptide of interest prior to step a), or conditioning the cleaved amino acid or the deletion fragment prior to mass spectrometry.

81. The process of claim 80, wherein the conditioning comprises reducing the charge heterogeneity of the polypeptide, the cleaved amino acid, or the deletion fragment.

82. The process of claim 81, wherein the conditioning comprises contacting the target polypeptide with a cation exchange material.

83. The process of claim 80, wherein the conditioning comprises mass modifying the polypeptide, the cleaved amino acid, or the deletion fragment.

84. The process of claim 80, wherein the agent is a chemical agent.

85. The process of claim 78, wherein the agent is an enzyme.

86. The process of claim 85, wherein the enzyme is an aminopeptidase or a carboxypeptidase.

87. The process of claim 78, wherein the polypeptide of interest is immobilized on a solid support.

88. The process of claim 87, wherein the solid support is selected from the group consisting of a bead and a microchip.

89. A process for determining the amino acid sequence of a polypeptide of interest using mass spectrometry, comprising the steps of:

a) producing a nested set of deletion fragments of the polypeptide; and

b) subjecting the deletion fragments to mass spectrometry, thereby determining the amino acid sequence of the polypeptide.

90. The process of claim 89, wherein the polypeptide of interest is immobilized on a solid support prior to producing the nested set of deletion fragments.

91. The process of claim 90, wherein the polypeptide of interest is immobilized to the solid support through a cleavable linker.

92. The process of claim 91, wherein the cleavable linker is selected from the group consisting of an acid cleavable linker and photocleavable linker.

93. A process for determining the amino acid sequence of each polypeptide in a plurality of polypeptides using mass spectroscopy, comprising the steps of:

a) differentially mass modifying each polypeptide in the plurality to produce differentially mass modified polypeptides;

b) contacting the differentially mass modified polypeptides with an agent that cleaves an amino acid from a terminus of the polypeptides to produce a cleaved amino acid and a deletion fragment;

c) subjecting the cleaved amino acid or the deletion fragment to mass spectrometry; and

d) repeating step b) and step c), as necessary, thereby determining the amino acid sequence of each polypeptide in the plurality.

94. The process of claim 92, wherein each polypeptide in the plurality is immobilized to the solid support.

95. The process of claim 94, wherein each polypeptide in the plurality is immobilized to the solid support through a cleavable linker.

96. The process of claim 95, wherein the cleavable linker is selected from the group consisting of an acid cleavable linker and photocleavable linker.

97. The process of claim 93, further comprising conditioning each polypeptide prior to step b), or conditioning the cleaved amino acid or the deletion fragment prior to mass spectrometry.

98. The process of claim 93, wherein the conditioning comprises contacting the target polypeptide with a cation exchange material.

99. The process of claim 93, wherein the agent is a chemical agent.

100. The process of claim 93, wherein the agent is an enzyme.

101. The process of claim 100, wherein the enzyme is an aminopeptidase or a carboxypeptidase.

102. The process of claim 93, wherein each polypeptide in the plurality is immobilized on a solid support.

103. The process of claim 102, wherein the each polypeptide is immobilized in an array.

104. A process for determining a nucleotide sequence of an unknown polynucleotide using mass spectrometry, comprising the steps of:

a) determining the amino acid sequence of a polypeptide encoded by the unknown polynucleotide by mass spectrometry by the method of claim 78;

b) comparing the amino acid sequence of the unknown polypeptide to an amino acid sequence encoded by a corresponding known polynucleotide, thereby determining the nucleotide sequence of the unknown polynucleotide.

105. The process of claim 104, further comprising conditioning the polypeptide encoded by the polynucleotide prior to contacting the polypeptide with an agent that cleaves an amino acid, or conditioning the cleaved amino acid or the deletion fragment prior to mass spectrometry.

106. The process of claim 104, wherein the polypeptide encoded by the polynucleotide is immobilized to a solid support.

107. A process for determining the identity of a target polypeptide, comprising the steps of:

a) obtaining the target polypeptide by *in vitro* translation, or by *in vitro* transcription followed by translation, of a nucleic acid encoding the target polypeptide;

b) contacting the target polypeptide with at least one agent that cleaves at least one peptide bond in the target polypeptide to produce peptide fragments of the target polypeptide;

c) determining the molecular mass of at least one of the peptide fragments of the target polypeptide by mass spectrometry; and

d) comparing the molecular mass of the peptide fragments of the target polypeptide with the molecular mass of peptide fragments of a corresponding known polypeptide, thereby determining the identity of the target polypeptide.

108. The process of claim 107, wherein the target polypeptide is immobilized to a solid support prior to contacting the target polypeptide with the agent.

109. The process of claim 107, wherein the target polypeptide is immobilized to the solid support through a cleavable linker.

110. The process of claim 110, wherein the target polypeptide is immobilized to the solid support through an chemically cleavable linker at one terminus of the polypeptide and through a photocleavable linker at the other terminus of the polypeptide.

111. The process of claim 107, wherein the target polypeptide is conditioned prior to step b), or the peptide fragments of the target polypeptide are conditioned prior to step c).

112. The process of claim 107, wherein the agent that cleaves at least one peptide bond in the target polypeptide is an endopeptidase.

113. A process for determining the identity of each target polypeptide in a plurality of target polypeptides, comprising the steps of:

a) obtaining a plurality of target polypeptides;

b) contacting each target polypeptide with at least one agent that cleaves at least one peptide bond in each target polypeptide to produce peptide fragments of each target polypeptide;

c) determining the molecular mass of at least one of the peptide fragments of each target polypeptide in the plurality by mass spectrometry; and

d) comparing the molecular mass of the peptide fragments of each target polypeptide with the molecular mass of peptide fragments of a corresponding known polypeptide, thereby determining the identity of each target polypeptide in the plurality.

114. The process of claim 113, wherein each target polypeptide is mass modified prior to step b), or the at least one peptide fragment of each target polypeptide is mass modified prior to step c).

115. The process of claim 113, wherein each target polypeptide in the plurality is immobilized to a solid support prior to contacting each target polypeptide with the agent.

116. The process of claim 115, wherein each target polypeptide is immobilized to the solid support through a cleavable linker.

117. The process of claim 113, wherein each target polypeptide is conditioned prior to step b), or the at least one peptide fragment of each target polypeptide is conditioned prior to step c).

118. The process of claim 115, wherein each target polypeptide is immobilized in an array.

119. The process of claim 113, wherein the agent that cleaves at least one peptide bond in each target polypeptide is an endopeptidase.

120. The process of claim 111, wherein each target polypeptide is immobilized to the solid support through a chemically cleavable linker at one terminus of the polypeptide and through a photocleavable linker at the other terminus of the polypeptide.

SEQUENCE LISTING

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<120> Mass Spectrometric Detection of Polypeptides

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Val His His His His His His
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(54) Title: MASS SPECTROMETRIC DETECTION OF POLYPEPTIDES

(57) Abstract

A process for determining the identity of a target polypeptide using mass spectroscopy is provided. Depending on the target polypeptide to be identified, a process as disclosed can be used, for example, to diagnose a genetic disease or chromosomal abnormality, a predisposition to a disease or condition, or infection by a pathogenic organism; or for determining identity or heredity. Kits for performing the disclosed processes also are provided.

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INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/US 98/18311

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 11249 A (GARVIN ALEX M) 19 March 1998 see page 4, paragraph 5 - page 5, paragraph 2 see page 6, line 4 - line 9 -/--	1-11, 17-28, 34, 38-44, 48,49, 52,55, 58-60, 63,64, 76,107, 108,111

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
11 June 1999	14. 07. 99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hart-Davis, J

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Int ıtional Application No
PCT/US 98/18311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X A	GB 2 168 478 A (SCAN LIMITED M) 18 June 1986 see the whole document	2,28,39, 40,58 78, 80-86, 89,93, 97-101, 104,105, 113,114, 117-119

X	WO 96 36732 A (UNIV ROCKEFELLER ;SCRIPPS RESEARCH INST (US); CIPHERGEN BIOSYSTEMS) 21 November 1996 cited in the application	2,28,39, 40,58
X	see page 5, line 32 - page 7, line 38	58,78, 80-83, 85,86, 89,93, 97,98, 100,101, 104,105, 113,114, 117-119

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X	see page 30; claims 1-10; figures 3,4	78, 80-84, 89,93, 97-99, 104,105, 113,114, 117,118

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INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/US 98/18311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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	see page 59, line 20 - page 60, line 25 see page 67, line 18 - page 68, line 11 see page 81, line 1 - page 84, line 6 see page 29, line 9 - page 33, line 10	

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	see page 28, line 12 - page 30, line 25; examples 4,5	

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INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 98/18311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 98/18311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	T KRISHNAMURTHY, P L ROSS: "Rapid identification of bacteria by direct matrix assisted laser desorption / ionization mass spectrometric analysis of whole cells" RAPID COMMUNICATIONS IN MASS SPECTROMETRY, vol. 10, no. 15, 1996, pages 1992-1996, XP002105701 see the whole document	2,28,39, 40,56-58

INTERNATIONAL SEARCH REPORT

Ir... ...national application No.
PCT/US 98/18311

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 98/18311

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1, 2 (in part), 3-13, 14-16 (in part), 17-27, 28-38 (in part), 39-40, 41-58 (in part), 59, 66-70

   Use of mass spectroscopy in the identification of polypeptides, the polypeptides having been obtained by prior translation of nucleic acids. Claims 14-16 and 28-57 have been searched in so far as they as they refer back to Claim 1 (these claims as filed depend on Claims 1 or 2).

2. Claims: 2 (in part), 14-16 (in part), 30-33 (in part)

   Use of mass spectroscopy in the identification of polypeptides, the polypeptide comprising a tag moiety. Claims 14-16 and 30-33 have been searched in so far as they as they refer back to Claim 2 (these claims as filed depend on Claims 1 or 2).

3. Claims: 2 (in part), 28-29 (in part), 34-38 (in part), 58 (in part), 60-65

   Use of mass spectroscopy in the identification of a polypeptide or of a plurality of polypeptides, the polypeptide(s) having been immobilized on a solid support prior to analysis by mass spectroscopy. Claims 34-38 have been searched in so far as they as they refer back to Claim 2 (these claims as filed depend on Claims 1 or 2).

4. Claims: 2 (in part), 41-57 (in part), 71-77

   Use of mass spectroscopy in the identification of polypeptides, the polypeptide(s) being associated with allelic variants and/or disease states. Claims 41-57 have been searched in so far as they as they refer back to Claim 2 (these claims as filed depend on Claims 1 or 2).

5. Claims: 78-120

   Use of mass spectroscopy in the identification of polypeptides, the polypeptide(s) having been treated prior to MS analysis by an agent which cleaves peptide bonds in the said polypeptides, thus producing peptide fragments.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 98/18311

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